Original Article

GENETIC VARIABILITY FOR EARLY RICE SEEDLING VIGOUR IN A F₃ POPULATION OF BPT5204/ IR88633-1-136-B2 UNDER DRY DIRECT SEEDED CONDITION

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ABSTRACT

In the present study the genetic variability among progenies of F_3 population segregating for ESVG (ESVG) was explored by phenotypic evaluation under dry-direct seeded system of rice cultivation. The F_3 population was derived from a cross between BPT5204/ IR88633-1-136-B2. IR88633-1-136-B2 is very good donor for ESVG. A new phenotyping method was standardised for screening ESVG under dry direct seeded condition. Analysis of variance and genetic estimates indicated there was significant genetic variation among progenies. Plant height, biomass and vigour index had high heritability and genetic advance at 15 and 30 days stages of crop, indicating that selection for ESVG will be effective at both stages of crop. Plant height, leaf number, tiller number, biomass and vigour index showed significant positive correlation with ESVG under both the stages of the crop. Families out-performing BPT5204 for ESVG progenies at 15 days and 30 days stages of the crop were obtained providing evidence that phenotypically IR88633-1-136-B2 contributed to ESVG increase. This donor can be a novel source of natural genetic variation for the improvement of rice under dry direct seeded condition of rice cultivation.

KEYWORDS: Genetic Variability, Early Seedling Vigour, Dry-Direct Seeded Rice, Transgressive Segregants

Received: Nov 24, 2015; Accepted: Dec 08, 2015; Published: Dec 11, 2015; Paper Id.: IJASRDEC201545

INTRODUCTION

Rice is a major source of food and income for half of the world's population. Worldwide various methods of rice cultivation have been adapted based on the availability of resources. In general, the traditional way of rice cultivation is done by germinating their seeds in nursery and grown by transplanting 3-4 week old seedlings into puddled fields. The advantages of the transplanted-puddled rice (TPR) system of crop establishment includes controlled weed growth (Surendra et al., 2001), easy seedling establishment (Farooq et al., 2011) and enhanced nutrient availability (e.g., iron, zinc, phosphorus) by creating an anaerobic condition. But, transplanting and puddling require a large amount of water, sufficient rainfall, labour and energy. Moreover, in the traditional TPR, puddling creates a hard pan below the plough-zone and reduces soil permeability. It leads to high losses of water through puddling, surface evaporation and percolation. Water resources, both surface and underground, are shrinking and water has become a limiting factor in rice production (Farooq et al., 2009). Huge water inputs, labour costs and labour requirements for TPR have reduced profit margins (Pandey and Velasco, 1999). As these factors are becoming limited, it makes rice production more expensive and less profitable (Mahajan et al., 2004).

Therefore, improving the rice productivity with economic security is a major challenge to develop an alternative solution. Hence, the situation demands a major shift in the rice cultivation system from TPR to direct-seeded rice (DSR). In recent years, there has been a shift from TPR to DSR cultivation in several countries of Southeast Asia (Pandey and Velasco, 2002). This shift was principally brought about by the expensive labour component for transplanting due to an acute farm labour shortage, which also delayed rice sowing (Chan and Nor, 1993).

Good crop establishment is a key factor for the success of subsequent crop growth. Direct seeding succeeds based on the topography of land, seedbed condition, oxygen level in the vicinity of germinating seed and the method of sowing. Therefore, direct seeding can be classified as (1) wet DSR (sprouted rice seeds are broadcast on wet soil); (2) dry DSR (dry rice seeds are broadcasted or drilled on dry soil) and (3) water DSR (seeds are broadcasted in standing water) (Mahender et al., 2015). Dry and wet seeding methods are popular among the rice farmers of rain fed lowland and gaining its place in irrigated ecology, as they require less labour and time than transplanting (Sarkar and Das, 2003). Farmers commonly practise wet seeding with pre-germinated seeds, where there is good control over the water supply. In areas where water supply is unpredictable, dry seeding is usually practised (Ella et al., 2011; Gathala et al., 2011). Compared to wet and water seeding, dry DSR is more advantageous in many situations, as it is less labour intensive, time saving in sowing the crop, consumes less water, suitable for lowland, crop matures 7-14 days earlier and there is less methane emission (Chauhan et al., 2012; Nguyen and Ferrero, 2006). Delay in transplanting reduces grain yield and seed quality because of poor seed set and biotic stresses due to high temperature and high humidity at flowering. Therefore, farmers tend to shift the crop establishment methods for lowland rice from transplanting to the direct seeding system (Joshi et al., 2013; Weerakoon et al., 2011). The adoption of a direct-seeded method for lowland rice culture would significantly decrease costs of rice production (Flinn and Mandac, 1986). To date, no specific varieties have been developed for this purpose. Existing varieties used for TPR do not appear to be well-adapted for seedling growth in an initially oxygen-depleted microenvironment. As a result, farmers often resort to the costly practice of increasing the seeding rate for DSR by 2-3 times. (Farooq et al., 2011).

Availability of efficient genotypes which respond to good and timely management practices determines the success of DSR. Rapid germination, emergence and early stage seedling vigour manifested in terms of better root and shoot growth are some of the important traits needed for DSR. Early vigour is a complex trait which imparts to rapid crop establishment and resource utilization. Genes promoting vigorous growth in young rice seedlings need to be identified and transferred into high-yielding cultivars. This will depend upon the identification of superior donors for seedling vigour and of traits that best predict field performance (Redona and Mackill, 1996). In our study we found that the popular variety BPT5204 has poor ESVG and IR88633-1-136-B2 has good ESVG in dry direct seeded condition. So, we have utilized BPT5204 as a recurrent parent and IR88633-1-136-B2 as a donor for ESVG. This is the first report of mapping population generated using IR88633-1-136-B2 as a donor for ESVG. In the present work to study the genetic variability of ESVG and the traits associated with it, we have generated an inbreed F₃ population derived from BPT5204/ IR88633-1-136-B2 to evaluate five vigour and its related traits under dry DSR condition.

MATERIALS AND METHODS

Location

The study was conducted at Barwale Foundation Research Farm, Maharajpet, Hyderabad, located at latitude of 17° 24' N and longitude of 78° 12' E, and an altitude of 536 m above mean sea level.

Plant Material and Population Development

BPT5204 a popular variety known for its fine grain type and cooking quality is used as a female parent and IR88633-1-136-B2 is used as donor for ESVG. The breeding procedure adopted in the population development is shown in Figure 1.

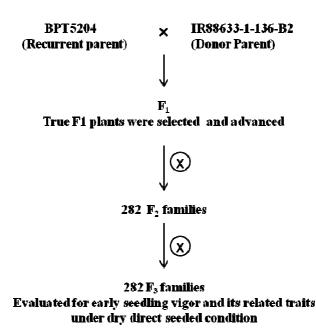


Figure 1: Flow Chart Showing the Breeding Method Adopted for the Development of F₃ BPT5204/ IR88633-1-136-B2 Mapping Population

True F_1 plants produced from the cross BPT5204/ IR88633-1-136-B2 were selected based on phenotype and confirmed genotypically using polymorphic simple sequence repeat (SSR) markers between the parents. Out of four true F_1 s identified, One F_1 was selected and selfed to derive 282 individual F_2 plants. Each individual F_2 plant was allowed to self to generate F_3 seeds. A total of 282 F_3 families were advanced for phenotyping without applying any selection pressure.

Field Trials

Evaluation of the mapping population along with recurrent parent was done under dry direct seeded condition, following a randomized block design with two replications during the wet season of 2012. In this trial, all 282 F₃ families along with recurrent and donor parents were sown in field directly into level, unpuddled, unflooded upland fields. Each family and recurrent parent and donor was sown in three rows of 10 plants each with a spacing of 20 cm between the plants and 20 cm between the rows (Figure 3.). The soil was amended with zinc sulphate at the rate of 2.5 kg ha⁻¹ before sowing. NPK fertilizer was applied at the rate of 80-40-40 kg ha⁻¹. N was applied in three equal splits at sowing, 40 days after sowing (DAS), and 60 DAS. Weeds were controlled by applying post-emergence herbicide Butaclor (3 L ha⁻¹), 10 DAS followed by manual hand weeding at later stages. Irrigation was given once in 4 days to maintain soil near field capacity till the germination of seedling to three-leaf stage.



Figure 2: Evaluation of the Mapping Population under Dry Direct Seeded Condition

Phenotypic Evaluation

Efficient screening techniques for evaluating breeding lines and identification of donors for high ESVG play crucial role to transfer of high ESVG trait in to high yielding popular varieties or hybrids. As on today there are no standard phenotyping methodologies for screening ESVG in rice under field conditions. So, we have developed a modified method based on the concepts of Yoshida (1981) given in his book 'Fundamentals of Rice Crop Science' and also based on the information provided in the book 'Standard Evaluation System for Rice' (1996) by International Rice Research Institute. We aimed on assessing the vegetative early two stages of the crop, viz., 15 days and 30 days after seeding, which are the most important stages for crop establishment in dry direct seeded condition of rice cultivation. Genotypes performing well with good ESVG at these stages were considered as high ESVG genotypes; those with medium ESVG were considered as medium ESVG genotypes and with poor ESVG were considered as poor seedling vigour genotypes.

Five plants were selected from the centre at random from each of the $282 \, \mathrm{F}_3$ families in each experiment and evaluated for the following 5 vigour-related traits:

Plant height (PH) measured in centimetres (cm) at 15 days (DS) and 30 days (DS) after germination of seedlings;

Leafs number (LN) measured as total number of leafs per plant at 15 DS and 30 DS after germination of seedlings;

Tiller number (TN) measured as total number of tillers per plant at 15 DS and 30 DS after germination of seedlings;

Biomass (BM) was calculated as the average weight of the five well-dried plants whose panicles had been removed;

Vigour index was calculated by multiplication of the plant height, number of leafs, number of tillers, with biomass at 15DS and 30 DS after germination of seedlings.

Statistical Analysis

Phenotypic data generated under dry direct seeded condition was analyzed without any transformation. Analysis of variance (ANOVA) was performed using AGRIS-GENRS software. Genotypic coefficient of variation (GCV),

phenotypic coefficient of variation (PCV), Heritability (h²), Genetic advance (GA) was derived using AGRIS-GENRS software.

RESULTS

Mean values of recurrent parent and population for ESVG and ESVG-related traits studied under dry direct seeded conditions are presented in Table 1.

Table 1: Mean Values of BPT5204 (Recurrent Parent, RP) and F₃ Population of BPT5204/ IR88633-1-136-B2 for ESVG and Related Traits under Dry Direct Seeded Conditions at 15 Days After Seeding and 30 Days after Seeding

Trait	RP Mean		Population Mean		F-Value	
	15 DS	30 DS	15 DS	30 DS	15 DS	30 DS
PH	16.7	22.1	18.3	30.3	1.7**	4.2**
LN	6.4	8.2	4.3	13.0	1.1	1.4**
TN	1.5	3.0	1.2	3.7	0.9	1.0
BM	153.5	153.5	207.9	207.9	4.3**	4.3**
VI	23893.0	83425.9	21058.5	314081.6	1.0	1.4**

^{*, **} Significant at 0.05 and 0.01 levels of probability, respectively, by F test

PH: plant height (cm), LN: leafs number, TN: tiller number, BM: biomass (g), VI: Vigour index.

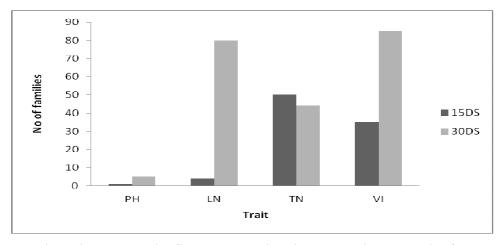


Figure 3: Transgressive Segregants obtained in the Mapping Population for ESVG Component Traits at 15Days and 30 Days Stages of Crop

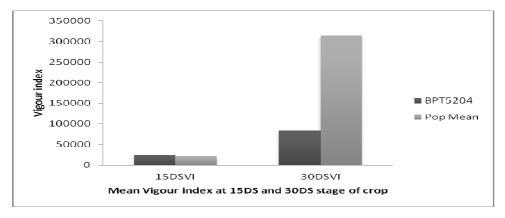


Figure 4: Comparison of Mean ESVG of the Recurrent Parent (BPT5204) and Mapping Population at 15 Days and 30 Days Stages of Crop

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The mean of the recurrent parent and population was slightly higher under 30 days stage of crop when compared to 15 days stage of crop for most of the traits. The VI of the recurrent parent was 83425.9 at 30 days stage of crop and 23893.0 at 15 days stage of crop with relative ESVG increase of 28.6% at 30 days stage of crop when compared to 15 days stage of crop. A similar kind of increase in mean values of all the traits was noticed in the F₃ population. However, several positive transgressive segregants out-performing the recurrent parent were observed for ESVG component traits under dry direct seeded conditions as shown in Figure 3. Comparison of mean ESVG of the recurrent parent (BPT5204) and mapping population at 15 days and 30 days stages of crop is presented in the form of an interaction plot in Figure 4 and it shows the ESVG of the population is significantly higher than the RP. There was significant variation in the population at 30 days stage of the crop for all the traits except for tiller number, where as there was significant variation for plant height, and biomass only at the 15 days stage of the crop as indicated by the results of ANOVA (Table 1).

Table 2: Estimates of GCV, PCV, h², and GA for ESVG and Related Traits in F₃ Population of BPT5204/ IR88633-1-136-B2 under Dry Direct Seeded Conditions at 15 Days after Seeding and 30 Days after Seeding

Trait	GCV		PCV		h^2		GA	
	15 DS	30 DS						
PH	10.7	13.0	21.3	16.6	0.25	0.61	11.1	21.0
LN	5.0	11.7	29.8	28.5	0.03	0.17	1.7	9.9
TN	6.8	4.3	34.7	28.9	0.04	0.02	0.8	1.3
BM	24.6	24.6	31.2	31.2	0.62	0.62	40.1	40.1
VI	16.3	29.6	44.6	72.7	0.10	0.17	3.8	24.9

PH: plant height (cm), LN: leafs number, TN: tiller number, BM: biomass (g), VI: Vigour index, GCV: genotypic coefficient of variation (%), PCV: phenotypic coefficient of variation (%), h²: broad sense heritability (%), GA: genetic advance as % of mean

In the present study, results revealed that the phenotypic coefficient of variation (PCV) was slightly higher than genotypic coefficient of variation (GCV) for all the characters studied indicating the presence of environmental influence in the phenotypic expression of characters (Table 2). At 15 DS stage GCV was the highest (24.6%) for biomass followed by vigour index (16.3%), the lowest GCV was observed for leaf number (5.0%) and at 30 DS. GCV was the highest (29.6%) for vigour index followed by biomass (16.3%), the lowest GCV was observed for Tiller number (4.3%), this indicates that there is higher degree of genetic variability among the population.

High to low h² and GA values were obtained for BM (0.62 and 40.1%) and PH (0.25 and 11.1%), at 15 DS, whereas at 30 DS, BM (0.62 and 40.1%), PH (0.61 and 21.0%) and VI (0.17 and 24.9%) recorded high to moderate values.

Association among ESVG Traits

An understanding of the nature of association among ESVG related traits namely, plant height, leaf number, tiller number, biomass and vigour index is expected to provide a better overview in understanding the relationship among ESVG traits. Correlation values among ESVG and ESVG-attributing traits are summarized in Table 3.

VI showed highly significant positive correlation with all the early stage seedling vigour-related traits at 15 DS and 30 DS stages of plants. A significant positive correlation was observed for VI with TN (r = 0.74), followed by LN (0.72), PH (0.68), and BM (0.43) at 15 DS. At 30 DS, LN (0.65) had the highest positive correlation with VI, followed by TN (0.63), BM (0.50) and PH (0.44). Among ESVG-related traits under both stages BM had highly significant negative association with LN followed by TN.

Table 3: Trait Correlation for ESVG and Related Traits in F₃ Population of BPT5204/ IR88633-1-136-B2 Traits under Dry Direct Seeded Conditions at 15 Days after Seeding and 30 Days after Seeding

	PH	LN	TN	BM	VI
PH (15 DS)					
PH (30 DS)					
LN (15 DS)	0.48**				
LN (30 DS)	-0.10				
TN (15 DS)	0.40**	0.90**			
TN (30 DS)	-0.14	0.93**			
BM (15 DS)	0.28**	-0.14*	-0.09		
BM (30 DS)	0.47**	-0.19**	-0.17*		
VI (15 DS)	0.68**	0.72**	0.74**	0.43**	
VI (30 DS)	0.44**	0.65**	0.63**	0.50**	

^{*, **} Significant at p = 0.05 and p = 0.01, respectively

PH: plant height (cm), LN: leaf number, TN: tiller number, BM: biomass (g), VI: Vigour index.

DISCUSSIONS

Genetic variation observed in the F_3 progenies of the advanced cross for ESVG and ESVG related components under dry direct seeded condition suggest evidence for the contribution of favourable alleles from IR88633-1-136-B2 for ESVG. The high-yielding transgressive segregants reported in this study are similar to earlier reports of obtaining positive transgressive segregants (Moncada et al., 2001; Septiningsih et al., 2003; Thomson et al., 2003; Marri et al., 2005; Swamy et al., 2011; Mohan et al., 2012).

Mean values of the population for all the ESVG component traits at 15 DS and 30 DS stage of the crop are significantly higher than the recurrent parent which suggests the influence of IR88633-1-136-B2 alleles in increasing the ESVG at both stages of crop. Transgressive families whose performance exceeded either of the parents in a positive direction were observed for ESVG and related traits at both stages of crop. These progenies are potentially useful breeding material for further studies as they serve as diverse germplasm for improving the ESVG of varieties and hybrids under dry direct seeded conditions.

In the present study, early seedling vigor related parameters, viz., plant height, leaf number, tiller number, biomass, and vigor index were assessed for variability and interrelationship individually in mapping population. Analysis of variance revealed highly significant differences among the population for ESVG and related traits studied, indicating presence of genetic variation.

The extent of environment influence on traits is explained by the magnitude of the difference between GCV and PCV. Large differences between GCV and PCV values reflect high environmental influence on the expression of traits (Seyoum et al., 2012). The results of this study revealed the greater role of genetic factors on the expression of traits. Similar results were reported by Seyoum et al. (2012); Mustafa and Elsheikh (2007); and Kole et al. (2008).

In the present study, plant height, biomass and vigor index exhibited high and moderate heritability at both the stages of the crop. High heritability indicates high component of heritable portion of variation, which can be utilized by breeders in selection of superior genotypes on the basis of phenotypic performance. High, moderate and low estimates of heritability were reported for different quantitative traits studied in rice (Seyoum et al., 2012; Khan et al. 2009; Hasan et al., 2011; Kole et al., 2008; Zahid et al., 2006).

Since high heritability does not always indicate high genetic gain; heritability estimates along with genetic advance would be more useful in predicting selection of superior genotypes (Seyoum et al., 2012; Hasan et al., 2011; Ali et al., 2002). In the present study leaf number and tiller number did not exhibited good genetic advance except but plant height, biomass and vigour index exhibited good genetic advance. Similar results were reported by Shenoy et al. (1990), Ramadevi (1998), Meli (2001), Bharathi et al. (2004) and Diwan et al. (2004) in different sets of rice genotypes indicated good scope of selection for different seedling vigor traits.

In trait correlations, PH (0.68 and 0.44), LN (0.72 and 0.65), TN (0.74 and 0.63), and BM (0.43 and 0.50) at 15 DS and 30 DS, respectively, followed a significant positive relationship with vigour index at 15 days and 30 days stage of the crop (Table 3). Hence, indirect selection of progenies based on these traits will also result in deriving lines with higher vigour index in advanced generations (Yuan et al. 2011) at both stages of crop. Similar results were reported by Cui et al. (2002) and Sujay (2007). Correlation patterns of PH and LN, PH and TN, differed significantly from 15 DS and 30 DS, indicating the role of G X E interaction on these traits.

CONCLUSIONS

The results from this investigation indicate that IR88633-1-136-B2 has high ESVG-enhancing alleles under dry direct seeded conditions. Hence, this donor can be a novel source of natural genetic variation for the improvement of rice under dry direct seeded conditions and simultaneously help in expansion of the cultivated gene pool of rice.

ACKNOWLEDGEMENTS

The authors are thankful to BMGF (Bill & Melinda Gates Foundation) and USAID (United States Agency for International Development) for financial assistance through CSISA (Cereal Systems Initiative for South Asia) project led by International Rice Research Institute (IRRI), Los Baños, Philippines. The authors are also thankful to management of Barwale Foundation, Hyderabad, India, for hosting IRRI-CSISA project and for providing all the facilities for execution of this research work.

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